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Developing Selenium-Enriched Animal Feed and Biofuel From Canola Planted for Managing Se-Laden Drainage Waters in the Westside of Central California

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DEVELOPING SELENIUM-ENRICHED ANIMAL FEED AND BIOFUEL FROM CANOLA PLANTED FOR MANAGING SE-LADEN DRAINAGE WATERS IN THE WESTSIDE OF CENTRAL CALIFORNIA

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We studied the reuse of selenium (Se)-laden effluent for producing canola (Brassica napus) and subsequent bioproducts in central California. Canola was irrigated with poor quality waters [electrical conductivity (EC) of \approx 5 dS m⁻¹ sulfate-salinity, 5 mg B L⁻¹, and 0.25 mg Se L⁻¹]. Typical seed yields were 2.2 metric tons ha⁻¹. Seeds were processed for their oil, and transesterified to produce ASTM-quality biodiesel (BD) blends. The resulting Se-enriched seed cake meal (containing \approx 2 mg Se kg⁻¹ DM) was used in a dairy feed trial. Seventy-two Jersey and Holstein cows, 36 respectively, were fed Se-enriched canola meal as 6.2% of their daily feed ration for five weeks. Blood and milk samples were collected weekly and analyzed for total Se. This study showed that Se-enriched canola meal did not significantly increase total blood Se content in either cow breed. Milk Se concentrations did, however, significantly increase to safe levels of 59 μ g Se L⁻¹ and 52 μ g Se L⁻¹ in Jersey and Holstein cows, respectively. The production of BD 20 biofuels and Se-enriched feed meal from canola irrigated with poor quality waters may help sustain similar phytomanagement strategies under Se-rich conditions.

KEYWORDS biofuel, selenium, phytoremediation, canola

INTRODUCTION

Interest in selenium (Se) pollution and remediation technology has escalated during the past two decades in Se rich regions of the western United States (Seiler *et al.*, 1999), China (Yang *et al.*, 2007) and India (Dhillon and Dhillon, 2003). Although not known to be essential for plants, selenium is essential for humans and animals and potentially toxic at high concentrations. A major Se controversy in the 1980s emerged in California when the general public and scientific community became aware of selenium's potential as an environmental contaminant. After extensive research on strategies to reduce loads of mobile Se from entering the drainage and agricultural water systems, a plant-based technology, defined as 'phytoremediation' received increasing recognition as

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a low-cost environmentally friendly approach for managing soluble Se in the soil and water environment. In this regard, researchers have demonstrated biological remediation strategies of Se under a variety of conditions (Dhillon and Dhillon, 2003; Bañuelos *et al.*, 2002; Lin *et al.*, 2002; Zayed *et al.*, 2000; Wu *et al.*, 2000; Frankenberger and Karlson, 1994, 1995).

Successful long-term field remediation of Se by plants is, however, dependent upon acceptance and widespread use by growers who are also concerned about the potential economical value from using a plant-based technology on their agricultural land. In this regard, Bañuelos (2002) reported that potential crops (e.g., broccoli, canola) used for the phytoextraction of Se in Central California, may produce agricultural products such as Se-enriched broccoli and Se-enriched animal feed. More recently, Bañuelos (2006) suggested processing seed for its oil from canola plants grown for the phytomanagement of Se. Vegetable oils from other oleaginous crops, e.g., sunflower (Helianthus annuus), safflower (Carthamus tinctorus), soybean (Glycine max), cotton (Gossypium hirsutum), and peanut (Arachis hypogaea) are considered to be potential alternative feedstocks for producing biofuels for diesel engines. Biofuels may offer partial relief from societal demand for petroleum, and their combustion products have the potential to be carbon neutral (Gomez et al., 2008), however, it is important that widespread production of biofuels does not occur from areas producing staple food crops.

Canola plants grown for the phytomanagement of Se under adverse soil conditions in the west side of the San Joaquin Valley (SJV) accumulate Se throughout the plant organs, including seed (Bañuelos, 2002). Bañuelos (2009) reports that after extracting oil from canola seed with an oil press, the residual seed meal contained Se at a concentration of 2 and 3 mg Se kg⁻¹ DM. The residual Se-enriched canola meal may be a convenient source of providing and ensuring adequate levels of Se in animal production, e.g., dairy and meat, since canola meal is one of the most widely traded protein ingredients in the world. Moreover, the added benefit from Se-enriched canola meal is that an organic source of Se may be a more bioavailable source of Se for maintaining healthy animal nutrition (Agbossamey et al., 1998; Bañuelos and Mayland, 2000). Selenium, while not required by plants, is an essential trace element for normal nutrition and health of animals (Mayland, 1994). Generally, animal diets containing 0.1 to 0.3 mg Se kg⁻¹ are adequate for Se animal nutrition (NRC, 1985; Mayland, 1994). It is a component of the enzyme glutathione peroxidase, an antioxidant capable of reducing cell-damaging free radical species produced during metabolism or from an oxidant stress (Gladyshev and Hatfield, 1999). Animal producers often times use inexpensive inorganic Se to ensure an adequate supply of Se to their livestock, although they are uncertain as to how much Se is actually absorbed and utilized by the animals.

Our objective was to determine the effectiveness of the derivation and novel utilization of two potential phytoproducts (biofuel and Se-enriched feed meal) from canola plants grown for the management and removal of Se in central California soils.

MATERIALS AND METHODS

A five-year study was conducted between 2002–2007 with *Brassica napus* var. Hyola (canola) on different 50-ha field sites at Red Rock Ranch (courtesy of Mr. John Diener, Five Points, CA). Canola was selected for its ability to accumulate Se from the soil under moderately high salinity and B levels (Bañuelos *et al.*, 1996). The soil in the experimental area was classified as an Oxalis silty clay loam (fine montmorillonitic, thermic Pachic

haploxeral with a well-developed salinity profile). The field site was drained by subsurface 15 cm diameter plastic drains, which were installed at depths ranging from 2.5–3 m with lateral spacing of 125 m.

Canola was directly seeded in late November of each year to a plant density of $100,000~ha^{-1}$ to take advantage of winter rains. Each bed contained two planted rows spaced 0.3~m apart. Two water sources were available for irrigation: canal water from the California aqueduct and Se-laden drainage water produced from other growing areas at Red Rock Ranch. The canal water had concentrations of Se $< 0.01~mg~L^{-1}$, $B < 1~mg~L^{-1}$, and a salinity (EC) $< 1~dS~m^{-1}$. The drainage water was pumped from a drainage sump, and had a range of Se from $0.100-0.150~mg~L^{-1}$, B from $4-7~mg~L^{-1}$, and a sodium sulfate-dominated salinity of $5-8~dS~m^{-1}$. Seeded fields initially received clean canal water via sprinkler irrigation until plant seedlings were deemed established (with true second or third leaves). Thereafter, furrow irrigation scheduling with drainage water was based upon evapotranspiration (ET) data reported from California Irrigation Management Information System (CIMIS) located at the University of California Westside Research Station (located less than 5~km from the field site).

Canola Oil Production

Canola plants were harvested generally 150 days after seeding when the pods first began to turn yellow. When 30-40% of the seeds were brownish-red in color, canola was swathed, and allowed to cure and ripen from 10–14 days in the swath before combining. When most seeds were mature with no visual green color, the seeds were collected and stored in tight storage bins. Canola seeds were then processed for its oil with a 'horizontal press' and 'extruder' (Insta-Pro, Int., Des Moines, IA) at an ideal rate of 20 tons of seeds per day at Red Rock Ranch. Canola seeds were put through screw presses or expellers ('horizontal press'), which mechanically removed the liquid oil from the seeds; then the residue (press cake) was further processed by the 'extruder' that utilized friction as the sole source of heat accompanied by pressure and attrition. This process ruptured remaining oil cells for the additional recovery of oil. The resulting "extrudate" was then pressed one more time for any unrecovered oil with the 'horizontal press.' All recovered oil was stored in airtight tanks, allowing for natural separation of canola oil and any remaining residual particles. Oil was then collected and transesterified (courtesy of Russel Teall, Biodiesel Industries, Santa Barbara, CA). The resulting canola cake meal was collected and stored at 5°C for future use in the dairy feed trial (described later).

Sub-samples were taken from canola cake meal and its quality was analyzed for Se and other nutritional parameters by A & L Analytical Laboratory, Modesto, CA; Great Lakes Scientific, Inc. Stevensville, MI; and Barrow-Agee Laboratories; see Table 1. Analysis of Se and other elements were carried out according to the method described by Bañuelos and Akohoue (1994) using an atomic absorption spectrophotometer (Thermo Jarrell Ash, Smith-Hieftje 1000, Franklin, MA) with an automatic vapor accessory (AVA 880) and inductively coupled plasma-mass spectrometer (ICP-MS Perkin Elmer Plasma 2000 Emission Spectrometer), respectively. External quality control standards for soils, and plant tissue samples were obtained from the National Institute of Standards and Technology (NIST). The standard used reference materials wheat flour (SRM 1567, with Se content of $1.1 \pm 0.2~\mu g~g^{-1}$ DM, and a 94% recovery), as well as an internal sediment standard collected from Kesterson Reservoir, CA (a total Se content of 7.5 and 25 mg kg⁻¹, and a 94% recovery), respectively. Moreover, random subsamples were taken from blood and

Table 1 Nutritional contents in canola seed meal after oil extraction

Elemental Analysis:	Se	В	Ca	Ċ	Cu	Fe	*	Mg	Mn	Мо	Na	ïZ	А	S	Zn
						1	mg kg ^{−1} DM								
	2.00 [†] (.3)	41 (5)	3855 (245)	0.1	4.7	184 (14)	7601 (512)	3147 (187)	39 (4)	0.45	(69)	0.55	6661 (498)	4643 (261)	39
Feed Quality Analysis ‡ :	ADF	Ash	CF	CP	Fiber	Lignin	Moisture	NDF	Nitrogen						
					%										
	20.0	3.5 (.2)	35.7 (2)	27.1	12.2	7.2 (1)	6.8	23.1 (2)	4.2 (.4)						
Vitamin Analysis:	Vitamin A	-	Vitamin D	Q	Vitamin E		Niacin		Riboflavin						
			$\mu \mathrm{g} \mathrm{kg}^{-1}$					mg kg ⁻¹							
	192 (14)		75 (4)		57		115 (6)		13 (1)						
Glucosinolates: $21\mu \text{ mol g}^{-1}$ (6)	$\begin{array}{c} 21\mu \bmod \S \\ (6) \end{array}$														

† Values represent the mean from 10 replications followed by the standard deviation in parenthesis collected over the five –year study.

‡ADF-Acid detergent fiber; CF-Crude fat; CP-Crude protein; NDF-Neutral detergent fiber. Typical values for alfalfa hay were as follows: ADF (24%), CF (30%), CP (24%), and NDF (37%) (analysis performed as described in methods and materials).

milk, and Se concentrations were validated by the California Animal Health and Food Safety Laboratory System at UC Davis, Davis, CA.

Animal Feed Trial

The canola seed meal feeding trial was conducted at the California State University (CSU) Fresno Dairy Unit using their modern dairy facilities to house the two different dairy cattle breeds—Holstein and Jersey. Treatments consisted of providing early- to midlactation, three- to four-year-old Holstein and Jersey cows with either Se-enriched canola seed meal (generated from Red Rock Ranch) or control-balanced feed rations. The control treatment included a commercially available canola meal (< 1 mg Se kg⁻¹). Two and 1.43 kg of control canola or Se-enriched canola seed meal was respectively mixed with 32 and 23 kg daily feed ration (consisting of alfalfa, corn, corn silage, cottonseed, and dried distillers grain) for Holstein and Jersey cows, on a daily basis (\approx 6.2% of their total daily intake). With the exception of the source of Se, the ingredients in the two feeds were identical. The cows were stored in 'free stalls,' or in four pens—two pens for Holstein cows (control and Se-treatment) and two pens for Jersey cows (control and Se-treatment). Each pen contained 18 cows. All cows were initially fed the control feed ration for three weeks. At the end of the third week, both Holstein and Jersey Se-treatment cows (18 respectively) received their daily feed ration with the Se-enriched canola meal for five weeks. At the end of the fifth week, all cows received the same control feed ration without Se-enriched canola meal. Blood and milk samples were collected weekly for a total of 11 weeks. Milk samples were drawn and composited by individual cows in two milkings over a 24-hour period with a milking machine, while blood samples were collected via caudal venipuncture, stored at -20°C, and total blood Se was analyzed as described above with the ICP-MS.

RESULTS

Biodiesel Production

Canola seed yields generally ranged from a mean of 2 to a high of 3.7 metric tons (Mg) ha⁻¹ under field conditions. Canola seeds generally contain between 35–40% oil. Selenium was not detected in the oil. We processed up to one Mg of canola seed per hour (or approximately 18 Mg per day), with an extraction efficiency of almost 85%. With an estimated high seed yield of 3.7 Mg ha⁻¹ from canola grown on poor quality soils and/or irrigated with poor quality water, an estimated yield of 1295 liters of canola oil can be produced. After transesterification, this oil can be designated as 100% biodiesel (BD 100) made from canola oil, or 6,475 liters of BD 20 biodiesel (a mixture of 20% vegetable oil and 80% petrodiesel) per ha was achieved.

The BD 20 (a typical and most commercially often sold blend) was successfully used to power 2003-model Cummins 5.9 L non-road, diesel irrigation booster pumps under load (1400 rpm, while irrigating 5 ha of almond trees) at Red Rock Ranch for over 100 hours. Preliminary emission analysis showed that carbon monoxide (CO), carbon dioxide (CO₂), and total hydrocarbons (THC) were reduced, while NO_x levels were still problematic (preliminary data noted in discussion). After extraction of oil from seed, the residual seed meal was ready to be utilized in the dairy feed trial.

Selenium-Enriched Dairy Feed Trial

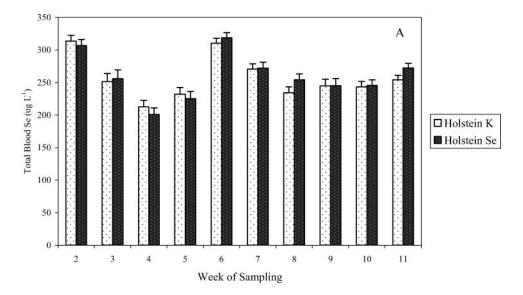
Results in Table 1 show the Se and nutritional content for the canola meal. These values are more than adequate when they are compared to the reported values for grass and or legume forage (NRC, 1985). More importantly, the Se concentration in the meal was at least 2 mg kg $^{-1}$ DM. The ADF (acid detergent fiber; 20%) and NDF (neutral detergent fiber; 23%) values were lower than those values for legume, grass and legume grass hays and silages (NRC, 1985). However, the CP (crude protein; 27%) and CF (crude fat; 21%) values were slightly better than legume hays. The low glucosinolate content in the meal of 21μ mol g $^{-1}$ was an important quality of the seed meal (Table 1).

Total blood Se values were not significantly increased by the feeding of Se-enriched canola meal ration to either cow breed (Figure 1), although blood Se values were elevated in Se treated cows compared to control cows at the end at the last feeding of Se-enriched meal (Holstein, 254 vs. 234 μ g Se L⁻¹ and Jersey, 252 vs. 234 μ g L⁻¹) and at the termination of the study (Holstein, 272 vs. 253 μ g Se L⁻¹ and Jersey, 237 vs. 230 μ g Se L⁻¹). Milk Se concentrations did, however, increase significantly in both cow breeds during the 5 weeks of receiving Se-enriched meal (Figure 2). Milk Se values ranged from 35–58 μ g L⁻¹ in Jersey cows compared to 34–52 μ g L⁻¹ in the Holstein cows that received the Se-enriched canola meal. During the fourth and eight week of milk sampling, milk Se concentrations increased on the average of 19% (from a range of 9–23%) and 14% (from a range of 2–21%) in Holstein and Jersey cows, respectively, when fed Se-enriched canola seed meal.

DISCUSSION

Reusing Se-laden drainage water on a crop such as canola can be one practical option for managing Se-laden drainage water or saline ground water in some western soils of central California (Ayars and Meek, 1994). The high sulfate concentration in this drainage water will inhibit the plant uptake of the chemical analogous of soluble selenate (Bell *et al.*, 1992). Hence, plant parts, including seed, rarely have concentrations of Se in excess of 5 mg kg⁻¹ DM (Bañuelos, 2002). This observed result almost ensures that Se-enriched seed meal produced from plants grown in the Westside of the SJV with a mean concentration of \approx 2 mg kg⁻¹ DM will not provide dairy cows, excessive Se when it is provided as 6.2% of the cow's daily feed ration (NRC, 1985).

Presently, the Red Rock Ranch (1875 hectares in size) consumes about 950,000 liters of diesel per year on site. The production and use of Red Rock- produced BD 20 biofuel could reduce their diesel consumption by 20%. The reduction of diesel usage will certainly add a commodity benefit to the Se phytomanagement strategy employed on drainage waters at the Red Rock Ranch site, as well as contribute to improving the overall air quality in the SJV, if practiced on a large scale (Bañuelos, unpublished data). In this regard, preliminary testing of the canola-based BD 20 biofuel under field conditions on the Cummins 5.9 L diesel irrigation booster pumps used at Red Rock Ranch, showed average reductions of CO (14%), CO₂ (13%), and THC (11%) compared to emissions emitted from the same booster pumps operating on petro diesel Reductions in NO_x values were not clearly observed. In light of the extremely poor air quality in the central SJV, a decrease in petrodiesel consumption and a potential decrease in emissions of CO, CO₂, and THC could give the agricultural community the first-hand ability to reduce air pollution by partially switching from petrodiesel to blended biodiesel in irrigation booster pumps



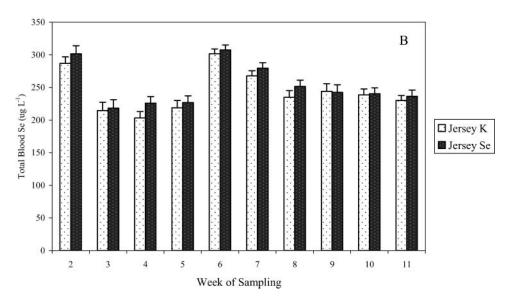


Figure 1 Selenium concentrations in whole blood collected on a weekly basis in (A) Holstein and in (B) Jersey cows. Values represent the mean from 18 replicates with the standard error bar. Means followed by the same letter are not significantly different according to Duncan's multiple range test at P < 0.05 level for each respective week. There were insignificant differences at any week of sampling in Se content between control-fed cows (K) and Se- enriched meal fed cows (Se) from both Holstein and Jersey breeds.

(note: there are over 5000 such pumps in operation in central California; Zoldoske, personal communication). Producing oil for biofuel from canola grown on poor quality soils and/or with poor quality water may become a significant byproduct process that contributes overall to the sustainability of field phytoremediation of Se.

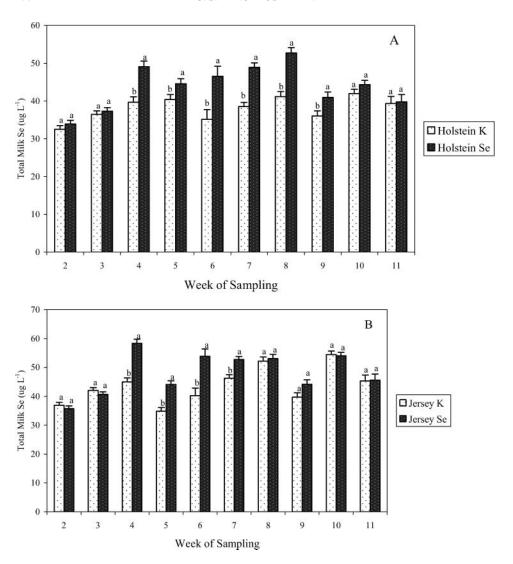


Figure 2 Selenium concentrations in milk collected on a weekly basis in (A) Holstein and in (B) Jersey cows. Values represent the mean from 18 replicates with the standard error bar. Means followed by the same letter are not significantly different according to Duncan's multiple range test at P < 0.05 level for each respective week between the control-fed cows (K) and the Se-enriched meal fed cows (Se).

Animal Feed Supplement

In addition to producing oil for biofuel production, Se-enriched canola seed meal, the major by-products resulting from the oil extraction processes of seed crushing, pressing, and extruding, is of high nutritional quality for use as an plant source of Se in the feed ration for lactating cows. As part of our phytoremediation process, we have produced Se-biofortified canola seed meal that should have an estimated higher cash value than most non-seleniferous canola meal. Moreover, this Se-enriched canola meal typically has Se concentrations ranging from a mean of 2 to a high of 3 mg kg⁻¹ when grown on

high sulfate salinity soil on the Westside of the SJV. The residual production of this Se-enriched meal may be of special importance for providing a plant source of Se to central California's livestock and dairy industries. Importantly, we were able to successfully increase the milk Se concentrations by as much as 23 and 21% in both Holstein and Jersey cows respectively, with the addition of Se-enriched seed meal into their daily feed rations for only 5 weeks. Generally, the detected milk Se levels in both control and Setreated cows were greater than most values reported by others, e.g., $17 \mu g L^{-1}$ (Pehrson et al., 1999); $20 \mu g L^{-1}$ (Gierus et al., 2002), and $31 \mu g L^{-1}$ Ortman and Pehrson, 1999), although their Se sources (e.g., yeast, grasses) contained Se concentration less than those concentrations contained within our canola seed meal. Milk Se concentrations may also vary depending on cow breed, as we detected between Holstein and Jersey cows. Importantly, our highest milk Se concentrations were still considered safe and above adequate for human consumption.

Our dairy findings concur with those previously observed by Muñez-Naveiro *et al.* (2006); it is possible to enrich cows Se content in milk with a plant source of Se. This observed increase in milk Se concentrations with plant material containing Se is consistent with other studies where Se levels increased more significantly from plant-like sources (*e.g.*, yeast, grass) than inorganic sources (*e.g.*, selenite) (Gierus *et al.*, 2002; Pehrson *et al.*, 1999; Ortman and Pehrson, 1999). Furthermore, Se in milk may be more bioavailable when provided to the cows as a plant source of Se, i.e., canola meal, than other inorganic sources of Se (Muñez-Naveiro *et al.*, 2006). The quality of milk, milk production, milk fat, protein, solids-not-fat, and somatic cell counts were not affected significantly with the use of Se-enriched canola meal in this study (data not presented). Enhancing the Se content of milk could be of more benefit for human nutrition, especially in Se-deficient regions, *e.g.*, Hubei regions of China (Yang *et al.*, 2007). Economics associated with transporting Se-enriched milk to Se-deficient regions would, determine the feasibility of such redistribution endeavors however.

Overall, total blood Se concentration, irrespective of treatment, were more than adequate at any sampling time (Gierus et al., 2002). Average total blood Se concentrations have been reported to be as low as 0.87 μ mole L⁻¹ (Pavlata et al., 2004) and 146 μ g L⁻¹ to achieve the optimal resistance against infectious mastitis (note: Se serum or plasma results were multiplied by 2 to give estimation of whole blood values) (Jukola, 1994; Olson, 1994). The observed increases in total Se content in blood were not consistent and significant from either cow breed fed the Se-enriched canola meal, despite the elevated blood Se concentrations detected (with, however, a large standard deviation) from treated cows. We presume the short-duration of the feed trial with Se-enriched meal, as well as the dilution effect of the whole blood analysis, prevented us from accurately determining any consistent and increased response of the Se accumulation in the blood. Similarly, other studies have shown that when blood plasma Se levels are already high (similar to total blood Se in this study), that additional Se supplementation does not increase Se levels further (Gierus et al., 2002). In contrast, selenium supplementation increases total and plasma blood Se in Se-deficient lactating ruminants (Ortman and Pehrson, 1999; Kessler and Lanz, 1995; Knowles et al., 1999). Moreover, since we did not analyze the excreta and urine from the cows, we were unable to quantify the amount of Se excreted by the cows (note: use of Se isotopes would be useful for this future determination). We assumed that an unknown quantity was excreted based upon another dairy feed trial with Se-enriched canola leaf material, where Bañuelos and Mayland (2000) detected concentrations as high as 73 μ g kg⁻¹ DM in manure and 59 μ g L⁻¹ in urine. Longer term studies with Se-enriched

feed are probably needed to evaluate the Se content in the cow's excreta more accurately. Because the estimated number of dairy cows is estimated to be over 1.8 million head in California alone, it is imperative to understand the potential concern to the environment with excessive Se-laden excreta. This quantity of Se could be dependent on the form of Se present in the original feed material.

In native forage sources of Se, the organically bound Se usually occurs as selenomethionine, which is absorbed by the same pathway as methionine (Ardüeser *et al.*, 1986). Future studies should include the identification and quantification of the organic forms of Se residing in the Se-enriched feed material. Selenium in the form of a selenomethionine may accumulate similarly to methionine (up to 2%); Bell (1995) in canola meal and be quickly incorporated into the hemoglobin. Measuring Se in blood serum and utilizing this information for determining the Se status in dairy cows has been discussed elsewhere (Maas *et al.*, 1992). In this regard, we will examine Se content in the blood serum with our on-going long term early to mid-lactation feed trial with Holstein and Jersey cows. Although bioavailability of organic Se supplied as Se-enriched seed meal and its subsequent absorption by animal tissues should be greater than inorganic forms of Se (Agbossamey *et al.*, 1998; Bañuelos and Mayland, 2000), Se absorption will always depend on type of animal (*e.g.*, cow versus sheep), early or late lactation stage, and dominant organic species of Se (*e.g.*, selenomethionine) contained within the organic matrix of canola seed meal.

CONCLUSION

The coupling of phytoremediation with biofuel production and animal feed enrichment products, *e.g.*, Se-enriched meal, can provide growers with a unique and realistic opportunity to increase environmental and economic sustainability in areas with high Se conditions. Developing successful phytomanagement strategies for Se is dependent on selecting plants or crop rotations that are most effective for removing or stabilizing the potential contaminant (Se) from the soil or waters over a long period. When possible, potential plant candidates should also be evaluated for the ability to produce products from non-food producing soils that may have economical value as a food supplement, soil conditioner, fuel additive, or other economical resource.

If this multi-faceted phytoremediation system proves to be sustainable on large land areas and is acceptable to growers, there could be long term economic benefits, as well as improved water and air quality associated with agricultural production in such Se rich regions. Moreover, sustained operation of this agronomic based system demonstrates the effectiveness of attempting to develop a holistic phytoremediation system for managing Se contamination and transforming them into valuable biofortified products and byproducts. Future studies should also include monitoring livestock manure for excessive Se since it can be a valuable resource, as well as a potential hazard to the environment.

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